

STORAGE STUDIES OF COW AND CAPRINE MILK DAHI INCORPORATED WITH α_s -CASEIN BIOACTIVE PEPTIDES

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ABSTRACT

The experiment was carried out on Dahi (curd) to assess the quality of Dahi prepared from cow and caprine milk. The cow samples were collected from Dairy farm, KVAFSU, Hebbal, Bengaluru and caprine milk samples were collected from Sinchana goat and sheep farm, Marenahalli village (Bengaluru Rural Dist) and Yashodhavana Goat Farm (Mysuru) were analyzed. There were different types of Dahi were prepared incorporating 0, 1.0, 1.5 and 2.0 per cent of bioactive peptides of caprine milk α_s -casein hydrolysates. The prepared Dahi samples were used to evaluate their quality of the Dahi were physical, chemical and microbiological. The different parameters of physical, chemical and microbiological were analyzed. The Dahi prepared from the cow was used as a control and the caprine milk Dahi was incorporated with different level of α_s -casein hydrolyzed Bioactive Peptides (BAP's). It was found that the caprine milk Dahi incorporated with α_s - casein hydrolysates of BAPs were optimized to the level of 1.5 per cent were found statistically significant ($p < 0.05$) difference with control and other samples. It extends their shelf life up to 15 days compared to control was 12 days under refrigeration condition ($5 \pm 1^\circ \text{C}$).

KEYWORDS: Caprine Milk, Dahi Quality, Casein Hydrolysates, Bioactive Peptides, Physical, Chemical & Microbiological Test

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INTRODUCTION

Dahi is a fermented Indian dairy product consumed by a larger section of peoples throughout the country, either as a part of the daily diet or as the refreshing beverage. About 7 per cent of total milk production is used for Dahi making. Dahi is produced by using mixed mesophilic cultures of *Lactococcus lactis* ssp *lactis*, *L. lactis* ssp *cremoris*, *L. lactis* ssp *diacetylactis* along with *Lecunostoc* species and lactose fermenting yeasts. Dahi is

well recognized for their therapeutic properties, particularly in curing gastrointestinal disorders [11].

Bio-Active Peptides (BAPs) obtained by urea filtration are hydrolyzed by enzymatic hydrolysis and gastrointestinal digestion plays an important role in metabolic regulation. The released peptides can be easily incorporated into fermented milk products to perform many vital physiological functions viz., anti-hypertensive, anti-oxidant, anti-cancer, anti-microbial, opioid activities, anti-oxidative and immune modulatory [13].

Further, an addition of hydrolysate enhanced the growth of *S. thermophilus* and acidification, thus reducing the fermentation time, and growth of probiotic bacteria. On the other hand, survival of probiotic bacteria was improved when milk was added with hydrolysate (0.25 to 4 g L⁻¹). As a consequence, the addition of hydrolysate had a differential action on probiotic cell counts in fermented milk after storage, depending on the balance between its negative effect on the growth of probiotic bacteria and its positive effect on the survival of probiotic bacteria [7].

The sensory evaluation of the goat milk with/without transglutaminase (TGase, at ranges of 0–4 units/g protein), TGase treated Labneh (a concentrated type of yogurt) the sensorial evaluation showed that significant differences ($P < 0.05$) were observed among samples for appearance, brightness, texture, odor, flavor, and consistence [1].

MATERIALS AND METHODS

Milk Samples

The indigenous and exotic caprine breed milk samples were collected from Sinchana goat and sheep farm, Marenahalli village (Bengaluru Rural Dist) and Yashodhavana Goat Farm (Mysuru) and cow milk samples were collected from Dairy Farm, Hebbal, KVAFSU Bengaluru.

Starter Culture

The mixed starter culture consisting of *Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*, *L. lactis* ssp. *diacetylactis* along with *Leuconostoc* species were procured from the NDRI, Audugodi, Bengaluru.

Trypsin Enzymes

2000 IU (Loba Chemicals Bovine Pancreas) a commercially available enzyme used for the hydrolysis of casein to obtain higher degree of hydrolysis (Enzyme substrate ratio of 1: 25).

Fractionation of Casein

The fractionation of casein from acid whole casein (wet) was followed as per the procedure of [5] on the basis of differential solubility in the urea solution. Fractionation of whole casein from 6.6 M urea to 4.63M urea yields a precipitate of α_s - casein.

Preparation of Casein Fraction Hydrolysates

The α_s -casein (CN) fractions of caprine milk were dispersed separately in distilled water at 40°C to give a 5 per cent (w/v) protein concentration and the pH of the solutions was adjusted to optimum as that of the enzymes using 0.1N NaOH. The enzyme trypsin (10 mg of enzyme/5 g of protein) at pH 8.0 and temperature 40°C was maintained. Enzymatic hydrolysis of casein fractions was carried out at an enzyme- substrate (E: S) ratio of 1:25 [12].

Degree of Hydrolysis

A Degree of Hydrolysis (DH) was determined by the pH-stat method [10], with slight modification in temperature and strength of alkali used to keep the pH constant during hydrolysis.

$$\text{Degree of Hydrolysis (DH)} = B \times N_b \times 1/\alpha_s \times 1/M_p \times 1 / h_{\text{tot}} \times 100$$

Where,

B= Base consumption in ml

Nb = Normality of Base (alkali)

Mp = Mass of protein in gram ($N \times fN$)

htot = Total number of peptide bonds in protein substrate (meq/g of protein for casein; htot = 8.2)

α = Average degree of dissociation of α - NH_2 groups $1/\alpha$ factor was considered.

$$\alpha_s = \frac{10^{\text{pH}-\text{pKa}}}{1+10^{\text{pH}-\text{pKa}}}$$

For casein $\text{pKa} = 7.45$ at pH 7.5, $1/\alpha = 1.89$.

Isolation of Bio-Active Peptides (BAPs)

The BAPs were isolated from α_s - casein fractions by adapting the method of [4], which is based on the principle that BAPs are soluble at pH 4.6 and aggregated with divalent cation such as calcium at neutral pH of 7.0. BAPs obtained by ethanol extraction were dried overnight in an oven maintained at a temperature of $70 \pm 1^\circ \text{C}$ and stored at 4°C before use. Hydrolysates of Casein fractions were subjected for centrifugation (3000 rpm/10 min). The obtained supernatant was adjusted to pH 7.0, then 1 per cent calcium chloride was added and kept for 1h. Ethanol (50 per cent v/v) was added to the supernatant to yield BAPs of α_s - casein fractions.

Quantification of BAPs from Casein Fractions

The quantification of BAPs from casein fraction was carried out by adapting the method suggested by [3].

Similarly, Dahi incorporated with different levels of BAPs (0, 1.0, 1.5 and 2 per cent) was analyzed for fat and total solid content were determined gravimetrically as per the reference procedure [2].

STATISTICAL ANALYSIS

Experimental data obtained in the study was analyzed by Randomized column block design as per the method described by [14] to test for 'F' values to know the statistical significance. Critical Difference (CD) value was calculated to determine whether the treatment means were similar or not. The analysis was done using SPSS software package and MS Excel 2007.

RESULTS AND DISCUSSIONS

Sensory Attributes of Caprine Milk Dahi Incorporated with α_s -Casein BAPs Stored at $5 \pm 1^\circ \text{C}$

Sensory attributes of caprine milk Dahi incorporated with α_s -casein BAPs stored at $5 \pm 1^\circ \text{C}$ are presented in Table

1.

The sensory score for all the samples decreased during the storage period. There was no significant difference ($p \geq 0.05$) on sensory attributes of color and appearance, body and texture and overall acceptability of caprine milk Dahi incorporated with different level of BAPs of the α -casein fraction. However, the flavor of caprine milk Dahi had a significant difference ($p \leq 0.05$) with respect to control. The sample C3 (1.5 % of α_{s-CN} BAPs) secured a significantly similar score of control, this was mainly because it retained the freshness of the product. All samples were unacceptable at the end of 12th day of storage due to surface discoloration, the higher level of syneresis and development of off-flavor, hence they are considered as unacceptable. Whereas sample (C3) found the development of off-flavor on 15th days of storage. These values are well within the range that was obtained by earlier workers [14] observed from Low-fat probiotic Dahi after 8 days of storage at 7°C. [9] was also noted that the addition of Whey Protein Concentrate (WPC) to a set-type fermented milk prepared from goat's milk had 12 days with high score appearance, taste, aroma, texture and overall acceptance and [8] was also observed unacceptable of goat milk yoghurt on 13th day of storage at refrigeration temperature ($4 \pm 1^\circ\text{C}$).

Table 1: Sensory Attributes of Caprine Milk Dahi Incorporated with α_s -Casein BAPs Stored at $5 \pm 1^\circ\text{C}$

Product	STORAGE DAYS																			
	Colour & Appearance					Body & Texture					Flavour					Overall Acceptability				
	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15
C0	8.50 ^a	8.35 ^a	8.25 ^a	NA	-	8.52 ^a	8.30 ^a	8.25 ^a	NA	-	8.58 ^a	8.12 ^a	8.26 ^b	NA	-	8.50 ^a	8.38 ^a	8.28 ^b	NA	-
C1	8.32 ^a	8.26 ^b	8.12 ^b			8.35 ^b	8.28 ^a	8.12 ^b			7.89 ^b	7.78 ^b	7.40 ^b			8.20 ^a	8.16 ^a	7.90 ^b		
C2	8.34 ^a	8.20 ^a	8.14 ^b			8.38 ^a	8.29 ^a	8.15 ^b			7.84 ^a	7.80 ^b	7.56 ^b			8.24 ^a	8.10 ^a	7.99 ^b		
C3	8.46 ^a	8.36 ^a	8.28 ^b	8.16 ^a	NA	8.46 ^a	8.39 ^a	8.29 ^a	8.12 ^a	NA	7.98 ^a	7.86 ^a	7.78 ^a	8.14 ^b	NA	8.36 ^a	8.28 ^a	8.29 ^a	8.17 ^a	NA
C4	8.35 ^a	8.28 ^a	8.18 ^b	NA	-	8.39 ^a	8.36 ^a	8.10 ^b	NA	-	7.89 ^b	7.82 ^b	7.29 ^b	NA	-	8.23 ^a	8.12 ^a	7.98 ^b	-	-
CD (P≤0.05)	0.48	0.56	0.42	-		0.36	0.42	0.23	-	-	0.023	0.042	0.028	-	-	0.36	0.45	0.065	-	-

#All the values are average of three trials

Similar superscripts indicates non-significance at the corresponding critical difference on a row

NA: Not Acceptable

C0: Control cow milk Dahi

C1: Caprine milk Dahi

C2: Dahi incorporated with α_s -Casein BAPs at 1.0 per cent level

C3: Dahi incorporated with α_s -Casein BAPs at 1.5 per cent level

C4: Dahi incorporated with α_s -Casein BAPs at 2.0 per cent level

Influence of α_s -Casein BAPs Levels on Chemical Quality of Caprine Milk DAHI Stored at $5 \pm 1^\circ\text{C}$

The α_s -CN BAPs incorporated (1.5%) to caprine milk pH stored at refrigeration temperature were 5.0, 5.0, 5.0, 5.0

and 4.8 and acidity were 0.67, 0.69, 0.70, 1.09 and 1.12 per cent lactic acid On 0, 3rd, 6th, 9th, 12th day of storage. Cow milk Dahi found not acceptable on the 12th day whereas α -casein BAPs incorporated (1.5%) found not acceptable on the 15th day at refrigeration condition are presented in Table 2. The obtained results in the present study are in agreement with the findings of [8] that the pH and acidity of caprine milk yoghurt stored at $4 \pm 1^\circ\text{C}$ was 4.8 and 1.05 per cent lactic acid and [6] reported on plain Dahi stored at refrigeration condition was analyzed for acidity and pH value. The pH of Dahi samples was decreased but the acidity percentage increased during storage at refrigeration temperature which was suitable for consumption up to 12 days.

Table 2: Chemical Quality Caprine Milk Dahi Incorporated with α_s -Casein BAPs Stored at $5 \pm 1^\circ\text{C}$

Sample	Storage Days									
	0		3		6		9		12	
	Acidity (%LA)	pH	Acidity (%LA)	pH	Acidity (%LA)	pH	Acidity (%LA)	pH	Acidity (%LA)	pH
C0	0.68 ^a	5.2 ^a	0.76 ^a	5.0 ^a	0.84 ^a	4.9 ^a	1.13 ^b	4.9 ^b	NA	-
C1	0.70 ^a	5.0 ^a	0.79 ^a	4.9 ^a	0.86 ^a	4.9 ^a	1.15 ^b	4.8 ^b	NA	-
C2	0.69 ^a	5.1 ^a	0.76 ^a	5.0 ^a	0.84 ^a	4.9 ^a	1.10 ^b	4.9 ^b	NA	-
C3	0.67 ^a	5.0 ^a	0.69 ^a	5.0 ^a	0.70 ^a	5.0 ^a	1.09 ^b	5.0 ^a	1.12 ^b	4.8 ^b
C4	0.70 ^a	5.1 ^a	0.70 ^a	5.0 ^a	0.87 ^a	5.0 ^a	1.12 ^b	4.9 ^b	NA	-
P value ($P \leq 0.05$)	0.023	0.036	0.046	0.041	0.063	0.061	0.021	0.025	-	-

#All the values are average of three trials

Similar superscripts indicate non-significance at the corresponding critical difference on a row

NA: Not Acceptable

C0: Control cow milk Dahi

C1: Caprine milk Dahi

C2: Dahi incorporated with α_s -Casein BAPs at 1.0 per cent level

C3: Dahi incorporated with α_s -Casein BAPs at 1.5 per cent level

C4: Dahi incorporated with α_s -Casein BAPs at 2.0 per cent level

Table 3: FFA and Soluble Nitrogen Content of Caprine Milk Dahi Incorporated with α_s -Casein BAPs Stored at $5 \pm 1^\circ\text{C}$

Products	Storage Days									
	0		3		6		9		12	
	FFA (% Oleic Acid)	Soluble Nitrogen (%)	FFA (% Oleic Acid)	Soluble Nitrogen (%)	FFA (% Oleic Acid)	Soluble Nitrogen (%)	FFA (% Oleic Acid)	Soluble Nitrogen (%)	FFA (% Oleic Acid)	Soluble Nitrogen (%)
C	5.2 ^a	0.09 ^a	11.50 ^a	0.14 ^a	11.58 ^a	0.21 ^a	12.45 ^a	0.26 ^b	Spoiled	-
C1	6.5 ^a	0.14 ^a	11.58 ^a	0.16 ^a	11.86 ^a	0.23 ^a	12.52 ^a	0.25 ^b		-
C2	6.7 ^a	0.16 ^a	11.56 ^a	0.18 ^a	11.94 ^a	0.25 ^a	12.86 ^a	0.26 ^b		-
C3	5.6 ^a	0.08 ^a	10.68 ^a	0.12 ^a	11.32 ^a	0.18 ^a	12.14 ^a	0.20 ^b	12.58 ^a	0.24 ^a
C4	6.4 ^a	0.10 ^a	11.52 ^a	0.13 ^a	11.84 ^a	0.26 ^a	12.83 ^a	0.25 ^b	spoiled	-
CD value ($P \leq 0.05$)	0.36	0.025	0.56	0.023	0.78	0.045	0.063	0.031	-	-

#All the values are average of three trials

Similar superscripts indicate non-significance at the corresponding critical difference on a row

C0: Control cow milk Dahi

C1: Caprine milk Dahi

C2: Dahi incorporated with α_s -Casein BAPs at 1.0 per cent level

C3: Dahi incorporated with α_s -Casein BAPs at 1.5 per cent level

C4: Dahi incorporated with α_s -Casein BAPs at 2.0 per cent level

Free Fatty Acids (FFA) and Soluble Nitrogen Content of Caprine Milk Dahi Incorporated with α_s -Casein BAPs Stored at $5 \pm 1^\circ \text{C}$

FFA and Soluble nitrogen content of caprine milk Dahi incorporated with α_s -casein BAPs stored at $5 \pm 1^\circ \text{C}$ are presented in Table 3. The development of Free Fatty Acid (FFA) value of oleic acid and soluble nitrogen content α_s -casein of 1.5 per cent BAPs level of caprine milk Dahi was 5.6, 10.68, 11.32, 12.14 and 12.58 per cent oleic acid respectively and soluble nitrogen content was 0.08, 0.12, 0.18, 0.20 and 0.24 per cent on 0, 3rd, 6th, 9th, 12th and 15th day of storage is presented in Table 3. The results are agreed with [8] for caprine milk yoghurt stored at refrigeration temperature ($4 \pm 1^\circ \text{C}$) had the shelf life of the 12th day.

Microbiological Quality of Caprine Milk Dahi Incorporated with α_s -Casein BAPs Stored at $5 \pm 1^\circ \text{C}$

The microbiological quality of caprine milk Dahi prepared from control and α_s -casein BAPs incorporated caprine Dahi did not show the presence of coliforms up to 12 days of storage. Whereas α_s -casein BAPs (1.5 %) incorporated caprine Dahi absent of coliforms and up to 15 days of storage under refrigeration condition. These results are in conformity with the findings of [8] revealed that goat milk yoghurt stored under refrigeration temperature ($4 \pm 1^\circ \text{C}$) for 12 days did show the presence of coliforms.

CONCLUSIONS

The sensory score Dahi of all the samples was decreased during the storage period. There was a significant difference ($p \leq 0.05$) on all sensory attributes of all the samples with respect to control. C3 sample (1.5 % α_s -CN BAPs) secured a significantly higher score than other samples this is mainly because it retained the freshness of the product. The development of acidity, release of free fatty acids and soluble nitrogen content were slowly found in C3 sample compared to control and other samples. The microbiological examination of Dahi reveals that the coliforms were absent during storage. All samples were unacceptable at the end of 12th day of storage due to surface discoloration, the higher level of syneresis and development of off-flavor, hence they are considered as unacceptable whereas C3 sample extends their shelf life up to 15 day of storage under refrigeration ($5 \pm 1^\circ \text{C}$). This may be due to slower acidity development, delay in the release of free fatty acids, the action of antibacterial and antioxidative activity of BAPs.

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